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Neuropeptide regulation of feeding in catfish, *Ictalurus punctatus*: a role for glucagon-like peptide-1 (GLP-1)?

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Abstract

Glucagon-like peptide 1 is a compound known to cause reduced food intake in mammals, though its action on feed intake in fish is unknown. The clear differences in the effects of GLP-1 on mammalian and teleostean glucose homeostasis suggest that we cannot assume a similar action of GLP-1 on feeding in mammals and fish. In this study the effects and specificity of centrally administered GLP-1 on feed intake were examined. It was demonstrated that intracerebroventricular (ICV) injection of glucagon-like peptide 1 (GLP-1) in the channel catfish (*Ictalurus punctatus*) is a potent inhibitor of feed intake with a dose of 0.25 ng g^{-1} body wt. reducing feed intake by 50%. The weak response to intraperitoneal (i.p.) and intravenous (i.v.) injection treatments with GLP-1 suggests the major effects on feed intake are centrally mediated. GLP-1 action on feed intake was not antagonized by ICV injection of exendin$_{9-39}$. Immunoneutralization of GLP-1 by ICV injection of antisalmon GLP-1 antisera did not affect feed intake over 48 h, while ICV injection of GLP-1 at a dose of 30 ng g^{-1} body wt. reduced feed intake for over 20 h. Additionally, there is some evidence that GLP-1 caused gastric evacuation. We conclude that GLP-1 is a potent inhibitor of feeding in fish, but its involvement in feed intake regulation under physiological conditions remains to be clarified. Published by Elsevier Science Inc.

Keywords: Feeding; Feed intake regulation; Glucagon-like peptide-1; Intracerebroventricular injection; Exendin$_{9-39}$; Immunoneutralization; Catfish; *Ictalurus punctatus*

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1. Introduction

The life histories of a number of fish species include prolonged periods of natural fasting (e.g. salmon *Oncorhynchus*, *Salmo salar*; sturgeon, *Acipenser*) and periods of gluttony (e.g. lamprey, *Petromyzon*; scorpion fish, *Scorpaena*). The incredible diversity of patterns with respect to feeding makes investigation of the regulation of feed intake in fish a rich area for comparative physiology. Smith (1999) outlined several criteria for demonstrating physiological function for a given peptide in feed intake: (1) behavioral specificity; (2) physiological dose range; (3) identification of the site of action; (4) type of receptor mechanism; and (5) ability to block actions with appropriate antagonists, antibodies or anti-sense molecules. Applying these criteria to peptides potentially regulating feed intake in fish has only begun.

One experimental technique to test physiological function is the direct intracerebroventricular (ICV) injection of peptides into the brain (de Pedro et al., 1993; Himick and Peter, 1994a,b; Silverstein and Plisetskaya, 2000). In addition, observations of gene expression under various treatment regimes, such as feeding and fasting are yielding information about genes involved with feeding and energy balance [Silverstein et al., 1998; Peyon et al., 1999, 2000; Narnaware et al., 2000, reviewed by Lin et al. (2000)]. These studies identify peptides that may have physiological relevance in regulation of feeding. Advances in understanding the regulation of feed intake in fish require that the physiological functions of peptides are clearly defined, and interactions between peptides identified.

GLP-1 is a member of the glucagon superfamily of peptides, and is encoded by the same preprohormone messenger RNA as glucagon (Andrews and Ronner, 1985). The amino acid sequence of channel catfish GLP-1_{1-31} was determined by Andrews and Ronner (1985) and shows approximately 68% identity with the human hormone and higher identity with other teleost GLP-1 proteins. The known actions of GLP-1 in fish and mammals for the regulation of glucose metabolism are remarkably different. In fish GLP-1 acts to stimulate gluconeogenesis and glycogenolysis (Mommsen et al., 1987) thereby increasing the pool of glucose, whereas in mammals it stimulates insulin release and slows gastric emptying (Mojsov, 2000) reducing glucose availability.

The role of GLP-1 in feed intake regulation has been investigated in mammals. The effect of ICV injection of GLP-1 is a strong inhibition of short-term feed intake (Turton et al., 1996a; Donahey et al. 1998). The physiological role of GLP-1 in regulating feed intake in the mammalian system is not certain, however.

In this study some of the criteria of Smith (1999) for determining physiological function in feed intake regulation were addressed for GLP-1. The dose response effects of ICV administration of GLP-1 on feed intake were investigated. Specificity of GLP-1 central action on feeding was examined by three additional experiments; peripheral administration of GLP-1, ICV injection of exendin$_{9-39}$, a potent GLP-1 antagonist in mammalian systems and ICV injection of antisera raised against salmon GLP-1, all followed by measurement of feed intake.

2. Materials and methods

The experimental fish were channel catfish (*Ictalurus punctatus*) obtained at the Stoneville, MS, facilities of the US Department of Agriculture, Catfish Genetics Research Unit. Fish were approximately 40–100 g, between 5 and 12 month of age, and maintained on well water in flow through 80-l tanks [temperature of approx. 26°C (pH 8.6), dissolved oxygen >5.0 mg l^{-1}, total hardness 51.3–68.4 mg l^{-1} as $CaCO_3$], 8–20 fish in each tank. Fish received a maintenance feed ration, they were fed to satiation between 08.30 and 10.30 h two to three times each week. In all experiments fish were fasted for 1 day prior to the start of an experiment, and injection and feeding experiments always took place between 08.30 and 12.00 h, unless otherwise specified. Two strains of catfish were used, USDA 103 and Norris [the origins and history of these fish are described in Li et al. (1998)].

Human glucagon-like peptide-1_{7-36}, (hGLP-1) with the amino acid sequence HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR was obtained from Peninsula Laboratories (Belmont, CA). Exendin$_{9-39}$, the truncated fragment of exendin-4, is an antagonist of the GLP-1 receptor isolated from the venom of the reticulate Gila monster (*Helo-*

derma suspectum) (Eng, 1992), and was purchased from Bachem (Torrance, CA). Synthetic channel catfish GLP-1_{1-31} (cfGLP-1) with the amino acid sequence HADGTYTSDVSSYLQDQAAKDFITWLKSGQP (Andrews and Ronner, 1985) was kindly provided by Dr Svetlana Mojsov of Rockefeller University (New York). All peptides were dissolved in phosphate buffered saline (PBS). Four separate experiments were conducted.

2.1. Experiment one — ICV injection of GLP-1

The effects of ICV injection with hGLP-1 and cfGLP-1 on feed intake were examined in fish from both Norris and USDA-103 strains. Doses ranging from 5 to 50 ng g^{-1} body wt. (BW) were dissolved in PBS and either PBS vehicle or GLP-1 was injected in a 2-μl volume into the third intracerebral ventricle of fish with an average weight of 57.0 ± 2.8 g (mean ± S.E.M.) following the procedure described in Silverstein and Plisetskaya (2000). Briefly, 12 fish at a time were anesthetized in a 0.01% solution of tricaine methanesulfonate (MS-222). Subsequently, when the fish were unconscious, one by one they were placed into a stereotactic device and injected ICV. Confirmation of the co-ordinates for needle placement was done previously by injecting methylene blue and observing the penetration of the dye throughout the ventricle (Silverstein and Plisetskaya, 2000). Lack of injury to the ventricle lining was demonstrated by microscopic evaluation of the lining following histological processing of the brain.

The dose response in feed intake was measured on fish from the USDA-103 strain following doses of cfGLP-1 ranging from 0.00 to 2.14 ng g^{-1} BW. Each dose was delivered to four fish, and there were eight control fish.

After injection, the fish were returned to their tanks and allowed 1 h to recover. Next, feed labeled with leaded glass ballotini beads (Sigmund Lindner GmbH, Warmensteinach, Germany) was delivered by hand over the following 1 h. In 15-min intervals, 10-g portions of labeled feed were introduced into the tank for a total of 40 g of feed (> 5% of BW). Subsequently, the fish were anesthetized (0.01% solution of MS-222), X-rayed and the amount of labeled feed consumed was calculated from the amount of label present in the stomach (Silverstein et al., 1999). Feed consumption was expressed as weight of feed consumed (g) 100 g^{-1} BW or % consumption. For comparison of treatments, mean consumption of controls was set to 100% and other treatments were expressed in relation to the control mean. ICV trials were conducted on several days and because there was no effect of treatment date, data were pooled. Consumption differences were evaluated by two-way ANOVA for strain and treatment effects, and one-way ANOVA was employed to examine the effects of dose on feed consumption. For all statistical tests $P \leq 0.05$ was considered significant.

2.2. Experiment two — i.v. and i.p. injection of GLP-1

PBS or cfGLP-1 dissolved in PBS was injected at a dose of 150 ng g^{-1} BW in 50-μl volume into the dorsal aorta along the roof of the mouth of fish from both Norris and USDA-103 strains ($n = 8$). Fish were first anesthetized in a 0.01% solution of MS-222, placed ventral side up in a surgical tray, and injected. Accuracy of needle placement was confirmed by pulling back gently on the syringe plunger to see that blood was pulled into the syringe prior to applying positive pressure to administer the GLP-1. Fish size did not differ between the strains and was 49.2 ± 4.1 g. For i.p. injections cfGLP-1 was injected as 100 ng g^{-1} BW in a 100-μl volume to fish of the Norris strain weighing 82.5 ± 4.3 g ($n = 6$). After injections, fish were returned to tanks and allowed to recover for 1 h. Labeled feed was then delivered to excess for 1 h, and consumption measured as above. The % consumption following i.v. injection was analyzed by two-way ANOVA (strain and treatment), and following i.p. injection data were analyzed by *t*-test (treatment).

2.3. Experiment three — ICV injection of exendin$_{9-39}$

An antagonist of GLP-1 action in mammalian systems, exendin$_{9-39}$, was tested in ICV injection on fish from the USDA-103 strain weighing 76.0 ± 3.3 g. Each independent trial consisted of four treatments, PBS control, 0.3 ng g^{-1} BW cfGLP-1, 12.5 ng g^{-1} exendin$_{9-39}$, and the combination of cfGLP-1 and exendin$_{9-39}$. One hour after the injection, labeled feed was presented to the fish and feed intake was measured as described above. Three trials were conducted and data pooled ($n = 14$). Consumption was reported relative to the

control. Treatment effects were analyzed by one-way ANOVA.

2.4. Experiment four — ICV injection with salmon GLP-1 antisera

Catfish of the USDA 103 strain weighing 41.6 $\pm$ 1.3 g, were ICV injected with 2 μl of either cfGLP-1 (30 ng g^{-1} BW), or antisera raised against salmon GLP-1 in rabbits, or PBS. This amount of antisera was shown to have a binding capacity of approximately 12–24 ng of GLP-1 in salmonids (Plisetskaya et al., 1989) and it has been used previously to measure GLP-1 plasma levels by radioimmunoassay in the related species *Ictalurus melas* (Ottolenghi and Plisetskaya, unpublished). Fish were allowed to recover for 1 h and labeled feed was delivered for 1 h ($n = 4$). Following a 30-min wait, fish were anesthetized, X-rayed and returned to their tanks. Feeding with labeled feed, anesthesia and X-ray were done next on a separate group at 3 h ($n = 4$) after injection. Subsequently, fish in both groups of tanks were given labeled feed, anesthetized and X-rayed at 8, 20 and 48 h ($n = 8$) after the initial injection. Feed consumption by fish in these treatments was compared by one-way ANOVA.

3. Results

3.1. ICV injection of GLP-1

ICV injection with all doses of GLP-1 greater than 5 ng g^{-1} BW were effective in blocking feeding almost entirely whether the ligand was hGLP-1 or cfGLP-1. Feed intake by controls, expressed as % consumption, ranged from 1.7 to 7.0% of BW in separate trials. USDA 103 strain catfish consumed significantly more feed than Norris strain fish ($7.0 \pm 0.5\%$ vs. $3.8 \pm 1.0\%$, $P < 0.0001$). Over all trials fish ICV injected with either hGLP-1 or cfGLP-1 had a mean % consumption of less than 0.1%. GLP-1 was effective in inhibiting feed intake in both strains.

A clear dose-dependent response in feeding was evident between doses from 0 to 0.54 ng g^{-1} BW (Fig. 1). Feed intake was significantly different between doses of 0, 0.26 and 0.54 ng g^{-1} BW ($P < 0.01$). A dose of 0.26 ng g^{-1} BW caused a 50% inhibition of feed intake.

3.2. i.v. and i.p. injection of GLP-1

The effect of an i.v. injection of cfGLP-1 on feed intake depended on the strain. Overall the USDA-103 strain fish consumed more feed than the Norris fish (6.6% vs. 2.9% BW, $P < 0.001$). Within the Norris strain GLP-1 i.v. treatment was associated with a 40% decrease ($P < 0.04$) in feeding (Fig. 2a). Feed intake of the USDA-103 fish was unchanged.

Only fish from the Norris strain were injected i.p. with cfGLP-1. There was no difference in feed consumed (2.0% BW, $P = 0.75$) between the GLP-1 injected and PBS injected fish (Fig. 2b).

3.3. ICV injection of exendin$_{3-39}$

The putative GLP-1 antagonist alone did not effect feed consumption. The doses of GLP-1 used were adequate to block feeding (Fig. 3a). When cfGLP-1 was injected in combination with exendin$_{9-39}$, the effect was the same as injection of cfGLP-1 alone, indicating that a 40-fold excess of exendin$_{9-39}$ could not antagonize the anorectic action of central GLP-1 in catfish.

3.4. ICV injection with salmon GLP-1 antisera

In this experiment, fish injected ICV with 30 ng g^{-1} BW hGLP-1 (7–36) did not consume feed until the 48-h sampling. Control fish and fish injected with the salmon GLP-1 antisera had a similar pattern of consumption. Feed in the stomach increased from 1 to 3 h after injection and

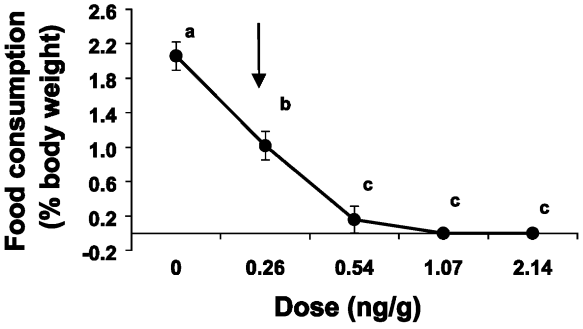

Fig. 1. Dose response in feed intake (% BW) to ICV injected cfGLP-1. Sample size was $n = 8$ for control and $n = 4$ for each dose. Points with different superscripts indicate significant difference at $P < 0.01$. The dose at which intake was 50% of controls is indicated by the arrow.

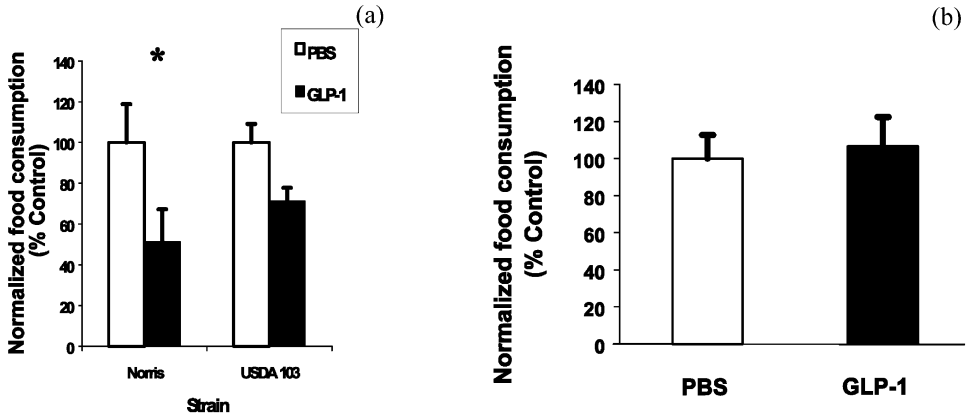

Fig. 2. (a) Feed intake following i.v. injection of cfGLP-1, 150 ng g^{-1} BW. The asterisk indicates a significant difference ($P < 0.04$) between cfGLP-1 injected Norris fish compared with their PBS injected counterparts, the difference in USDA-103 fish was not significant ($n = 8$). (b) Intraperitoneal injection of fish from the Norris strain with PBS or 100 ng g^{-1} BW cfGLP-1 ($n = 6$).

then declined between 3 and 20 h after injection (Fig. 3b). At 48 h all fish ate between 2.5 and 4.5% of BW. The GLP-1 treated fish ate significantly less through 20-h post-injection, however, there was no difference in consumption between PBS and salmon GLP-1 antisera treated fish. There were no significant treatment effects at 48 h.

4. Discussion

The potent inhibitory effect of centrally administered GLP-1 on feed intake in catfish is generally similar to the central effects demonstrated in mammals (Turton et al., 1996a). This similarity of central nervous system GLP-1 action in fish and mammals stands in contrast to large differences between piscine and mammalian responses to peripheral treatment with GLP-1 (Plisetskaya and Mommsen, 1996). Peripherally the major GLP-1 target tissues differ, being liver in fish and pancreas in mammals. The major action of this hormone also differs, stimulating glycogenolysis and gluconeogenesis in fish (Mommsen et al., 1987), vs. stimulating insulin release while slowing gastric emptying in mammals (Mojsov, 2000). Regardless of the dramatic differences in GLP-1 peripheral action, and because of the resemblance between fish and mammalian GLP-1 receptors and transduction mechanisms, Mommsen (2000) predicted that the central actions of GLP-1 may be similar between fish and mammals, and suggested that the functions of GLP-1 in the brain may be conserved throughout vertebrate evolution.

The dose of GLP-1 necessary to cause a 50% reduction of feed intake in catfish, of 0.25 ng g^{-1} BW or approximately 3×10^{-7} M (this assumes an intraventricular volume of 10–15 μl), is the lowest effective dose (approx. 4–500-fold less) reported for any ICV injected peptide in fish to date. GLP-1 may be one of the most potent anorexigenic peptides tested. Furthermore, the effective dose of GLP-1 to reduce feeding in rats was approximately 9 ng g^{-1} BW (van Dijk et al., 1997) suggesting that GLP-1 in catfish is nearly 40 times more potent than in rats. The effective dose for catfish is potentially in a physiological range, but still represents a large quantity of bioactive material. The possibility that we are observing pharmacological effects can not yet be ruled out.

The feed intake inhibiting action of GLP-1 appears to be mediated largely through the brain, as shown by the response in feed intake to ICV injection. The ICV injection, however, is still general in terms of which brain regions may be responding (van Dijk and Thiele, 1999). The dose is large enough to diffuse throughout the brain, particularly to any sites lining the brain ventricle. Infusion of peptides to discrete locations within the brain would be necessary to determine the specific brain nuclei responding, but such work has not been conducted in fish systems, yet. Intravenous injection of GLP-1 did cause reduced feeding in catfish, particularly the Norris strain of catfish. This effect may be due to the peptide crossing the blood–brain barrier (Olson et al.,

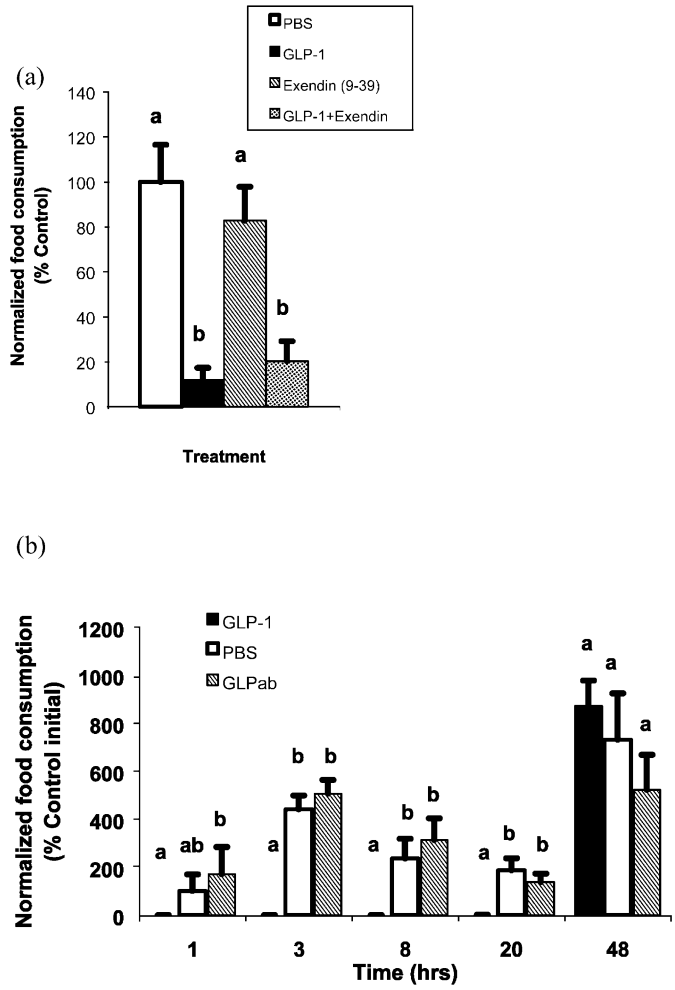

Fig. 3. (a) Feed consumption following ICV injection of PBS (control), cfGLP-1 (0.3 ng g^{-1} BW), the putative GLP-1 receptor antagonist exendin$_{9-39}$ (12.5 ng g^{-1} BW), and cfGLP-1 + exendin$_{9-39}$ (same doses as above). All treatments ($n = 14$) were administered in 2 μl volume. Feed consumption was normalized to controls. Bars with different superscripts are significantly different ($P < 0.005$). (b) Time course of feed consumption (normalized to control initial intake value) after ICV injection of hGLP-1 (30 ng g^{-1} BW), PBS and undiluted antisera against salmon GLP-1 (GLPab). All injections were in 2 μl volume. At times 1 and 3 h: $n = 4$, and at 8, 20 and 48 h: $n = 8$. Bars with different superscripts within a time point are significantly different ($P < 0.05$).

1978). Injection into the dorsal aorta would infuse a dose of GLP-1 just as the blood passes through the gill arches and makes its cranial circuit. The lack of response in feed intake to i.p. injection of GLP-1 suggests that the peptide was not able to make it to target tissues either in high enough concentration or fast enough before breakdown, to have an effect on feed intake. The higher concentration and relatively immediate delivery to the brain of GLP-1 may be the reason for the apparent effect of i.v. injection vs. no effect of i.p. injection. In mammals, i.v. administration of GLP-1 acts to slow gastric emptying and reduce food intake at doses up to 600-fold lower than tried in this study [reviewed by van Dijk and Thiele (1999)]. However, in rats i.p. doses similar to the i.p. dose used on catfish in this study did not appear to affect feeding in rats (Turton et al., 1996a). Higher doses may be tried in fish before ruling out a peripheral effect, but the anorexigenic effect of GLP-1 in the brain was much more potent than in the periphery.

The high sensitivity of response to GLP-1 suggests that inhibition of feeding is a receptor mediated event. Exendin$_{9-39}$ is a potent antagonist of GLP-1 action in mammals (Turton et al., 1996a,b; Meeran et al., 1999), so we expected the antagonist of the mammalian GLP-1 receptor to also

antagonize the fish GLP-1 receptor. Nevertheless, Mommsen (2000) reported that in a fish hepatocyte culture system, exendin$_{9-39}$ did not antagonize the physiological effect of GLP-1. Our results with ICV injection of exendin$_{9-39}$ are wholly consistent with this finding. Characterization of the transduction mechanism by which GLP-1 inhibits feeding in fish, awaits a specific antagonist of piscine GLP-1. It appears that GLP-1 in fish like in mammals, binds to a G coupled protein receptor (Mojsov, 2000), and causes activation of adenylyl cyclase (Mommsen and Mojsov, 1998). In mammals, both peripheral (at the pancreas) and central (at the brain) GLP-1 actions are transduced through the same type of receptor, so it is likely that fish also have a single GLP-1 receptor [Mojsov, 2000; but also see Montrose-Rafizadeh et al. (1997)], but it is evidently not blocked by the mammalian antagonist. Use of compounds that block intracellular cAMP mediated signal transduction may provide clues as to how GLP-1 acts in fish brain.

If GLP-1 plays a physiological role in reducing feed intake, immunoneutralization of endogenous GLP-1 would be expected to cause an increase in consumption. The attempt to immunoneutralize GLP-1 present in the brain with antisera produced against salmon GLP-1 was unsuccessful. The lack of response might be interpreted as a lack of endogenous GLP-1. The ability to measure plasma GLP-1 by radioimmunoassay using these same antibodies in the related species *Ictalurus melas* (Ottolenghi and Plisetskaya, unpublished) suggests that non-recognition of the antigen, or some other insufficiency of the antisera was not the problem. Mice with a disrupted GLP-1 receptor exhibited no abnormality in their feeding, leading Scrocchi et al. (1996) to suggest that GLP-1 signaling was not important for normal feeding behavior in mice. As mentioned above, identification of an antagonist, or some other technique to block endogenous hormone signaling such as treatment with an anti-sense oligo-nucleotide to block translation of functional GLP-1 is key to understanding the physiological role of GLP-1 in the fish brain (Turton et al., 1996b; Smith, 1999).

In a related preliminary study we noticed that while even after a 5-day fast, PBS injected fish had some feed residue in their gastro-intestinal (GI) tract, the GI tract of GLP-1 treated fish was entirely clear (Silverstein and Leonard, unpublished). It appeared that ICV injection of GLP-1 triggered an emptying of the GI tract. This evacuating effect of GLP-1 treatment is in contrast to the typical slowing of gastric emptying seen in mammals (van Dijk and Thiele, 1999) and raises questions about the potential pathological effect of GLP-1.

In summary, some of the criteria of Smith (1999) for demonstrating physiological function for a given peptide in feed intake regulation have been addressed, but not completely tested. The strong inhibitory activity of GLP-1 on feed intake was apparent, but behavioral specificity (criterion 1) is questionable because of the potential sickening affect of GLP-1. The required doses were within the possible physiological range (criterion 2). Central injection was far more effective than peripheral treatment suggesting that the site of action was in the brain (criterion 3, site of action). The unsuccessful attempts to antagonize or immunoneutralize GLP-1 effects (criterion 5) have left a gap in defining the receptor mechanism (criterion 4). Given the robust feeding inhibition caused by GLP-1 and the evacuation of the GI tract caused by treatment, the physiological role of central GLP-1 in feed intake regulation of fish remains uncertain. In mammals too, there is mounting evidence that GLP-1 causes reduced intake by making animals sick (Thiele et al., 1997; van Dijk and Thiele 1999). van Dijk and Thiele (1999) suggested that strong stimulation of central GLP-1 pathways in mammals may function to protect an animal that over-consumes a contaminated substance, and a more subtle stimulation of specific brain area may signal satiation. While GLP-1 and GLP-1 receptors are clearly expressed in fish brain (Busby and Mommsen, 2000), knowledge of their distribution and regional activity within the brain as well as appropriate inhibitors or knock out fish models will be required to clarify the role of GLP-1 in feed intake of fish.

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